

Sub A > **What is claimed is:**

1. A method of increasing or inducing cold or freezing tolerance in a plant, which comprises the following steps:

5 a) acclimating said plant to a temperature not lower than the coldest temperature that said plant is capable of withstanding, for a time sufficient to induce cold or freezing tolerance in said plant, and

b) increasing the concentration of betaine or a derivative thereof in said plant to induce cold or freezing tolerance in said plant;

10 whereby combined steps a) and b) increase or induce cold or freezing tolerance of said plant over and above the cold or freezing tolerance induced by each step alone.

2. A method as set forth in claim 1, wherein the increased or induced cold or freezing tolerance in said plant decreases the lethal temperature of said plant.

3. A method as set forth in claim 1, wherein the step of increasing the concentration of betaine or a derivative thereof includes administering a composition comprising betaine or a derivative thereof to said plant.

4. A method as set forth in claim 1, wherein the step of increasing the concentration of betaine or a derivative thereof includes overexpressing one or more genes involved in the synthesis of betaine or a derivative thereof.

20 5. A method as set forth in claim 4, wherein said one or more genes comprise betaine dehydrogenase or choline monooxygenase.

6. A method as set forth in claim 4, wherein said one or more genes are overexpressed under the control of a low temperature promoter.

7. A method as set forth in claim 1, wherein said temperature is higher than about 0°C.

25 8. A method as set forth in claim 1, wherein said temperature is below about 6°C.

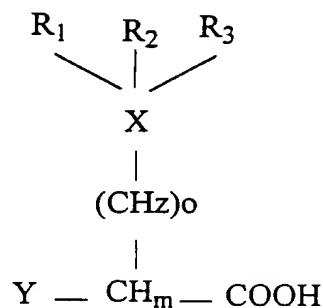
9. A method as set forth in claim 1, wherein steps a) and b) are substantially simultaneous.

10. A method as set forth in claim 1, wherein step b) precedes step a).

11. A method as set forth in claim 1, wherein step b) follows step a).

12. A method as set forth in claim 1, wherein said betaine or the derivative thereof comprises glycine betaine.

5 13. A method as set forth in claim 1, wherein said betaine or the derivative thereof comprises a compound of the formula



wherein X is N or S

z is 1 or 2

o is 0 or 1

10 m is 1 or 2

R₁, R₂, R₃ are independently void or hydrogen or methyl

Y is void or Q-CH_w-

wherein w is 1 or 2,

Q is a molecule of 2 to 9 carbon atoms comprising or not a ketone or a hydroxyl group, which may comprise 1 or 2 nitrogen or sulfur atoms and which may form a heterocycle alone or with X.

14. A method as set forth in claim 1, wherein said betaine or derivative thereof is selected from Glycinebetaine, β-alaninebetaine, 2-trimethylamino-6-ketoheptanoate,

20 prolinebetaine, proline, N-methyl-L-proline, *trans*-4-hydroxy-N-methyl-L-proline, *cis*-3-hydroxy-N-methyl-L-proline, (-)-4-hydroxyproline betaine, (+)-4-hydroxyprolinebetaine, 3-hydroxyprolinebetaine, histidinebetaine, tryptophanbetaine, 2-mercaptophistidinebetaine, pipecolabetaaine and nicotinic acid betaine.

15. A method as set forth in claim 1, wherein said plant is selected from the group consisting of tropical plants, gramineae and grasses.

16. A method as set forth in claim 15, wherein said plant is barley or wheat.
17. A method as set forth in claim 15, wherein said plant is golf turf.
18. A method as set forth in claim 10 or 11, wherein said composition comprises glycine betaine at a concentration lower than about 500 mM.
- 5 19. A method as set forth in claim 12, wherein said concentration is about 250 mM or lower.
20. A method as set forth in claim 13, wherein said concentration is about 250 mM.
21. A method as set forth in claim 17, wherein the method increases winter survival, spring regrowth, greening or density of golf turf.
- 10 22. A method as set forth in claim 20, wherein the increase in freezing tolerance is by at least about 5°C.
23. A method as set forth in claim 1, wherein the cold or freezing tolerance induced by said each step alone or in combination is due at least in part to an increased accumulation of a protein having the biological characteristics of WCOR410 protein.
- 15 24. A method as set forth in claim 1, which further results in improving photosynthetic capacity and overall physiology of said plant at cold temperature.
25. A method as set forth in claim 1, which further results in increasing or inducing tolerance of salinity, water stress, or both, in said plant.
- 20 26. A method of reducing the growth rate of a plant, thus having a growth-retarding effect, which comprises the step of treating said plant with an effective amount of a composition comprising betaine or derivative thereof, which composition is not lethal to said plant.
27. A method as set forth in claim 26, wherein said dosage regimen of betaine or derivative thereof is not toxic to said plant.
- 25 28. A method as set forth in claim 26 or 27, wherein said plant is selected from gramineae and grasses.

29. A method as set forth in claim 28, wherein said plant is golf turf.
30. A method as set forth in claim 28, wherein said plant is wheat or barley.
31. A method as set forth in claim 28, wherein said dosage regimen in growing said plant in the presence of betaine is at a concentration of 500 mM or lower for four days, which results in a growth rate reduction by about 75%.
32. A method of stimulating and improving the germination rate of plant seeds at a temperature not lower than the coldest temperature that said plant seeds can withstand, which comprises the steps of administering to said seeds an effective amount of a composition comprising betaine or a derivative thereof, and allowing said seeds to germinate at said temperature.
33. A method of inducing or increasing cold tolerance in a plant sensitive to temperature of about 0°C or higher, which comprises the step of administering a composition comprising betaine or a derivative thereof to said plant prior to a decrease in temperature.
34. A method of increasing or inducing cold or freezing tolerance in a plant, which comprises the step of increasing the accumulation of a protein having the biological characteristics of WCOR410 in said plant.
35. A method as set forth in claim 34, wherein said protein having the biological characteristics of WCOR410 is increased by increasing the concentration of betaine or a derivative thereof in said plant.
36. A method as set forth in any one of claims 1, 33 and 34, wherein each mention of said cold or freezing tolerance comprises about an optimal cold or freezing tolerance.
37. A method as set forth in any one of claims 23, 34 or 35, wherein the protein is WCOR410.

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